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Activating Mutations of the $G_s\alpha$ Gene Are Associated with Low Levels of Gsα Protein in Growth Hormone-Secreting Tumors*

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ABSTRACT

Evidence suggests the existence of a direct relationship between cellular $G_s\alpha$ content and activation of the adenylyl cyclase system. Data on $G_s\alpha$ levels in endocrine tumors that depend on cAMP for growth, particularly pituitary adenomas, are still limited. The levels of $G_s\alpha$ protein were evaluated in 11 GH-secreting adenomas with $G_s\alpha$ mutations (gsp^+) and 15 without (gsp). Complementary DNAs from gsp^+ tumors contained very low amounts of wild-type $G_s\alpha$ sequences, indicating a preponderance of the mutant $G_s\alpha$ transcripts in these tumors. Immunoblotting of $G_s\alpha$ protein showed that the two isoforms were present at high levels in all gsp^-, but were undetectable or barely detectable in gsp^+. The low $G_s\alpha$ content in gsp^+ tumors was not due to a reduction in ribonucleic acid synthesis or stability, as $G_s\alpha$ messenger ribonucleic acid levels were similar in wild-type and mutant tissues. Treatment of gsp^- cells with cholera toxin caused a marked reduction of $G_s\alpha$ levels. As in other cell systems cholera toxin increases $G_s\alpha$ degradation, our data are consistent with an accelerated removal of mutant $G_s\alpha$. This may represent an additional mechanism of feedback response to the constitutive activation of cAMP signaling in pituitary tumors with mutations in the $G_s\alpha$ gene. (*J Clin Endocrinol Metab* **83:** 4386–4390, 1998)

PITUITARY adenomas are monoclonal neoplasia due to genetic alterations that cause abnormal growth either by activating growth stimulatory proteins or by inactivating growth inhibitory signals (1). Although in recent years a large number of protooncogenes and antioncogenes have been screened for mutations in pituitary tumors, only loss of heterozygosity in 11q13 and mutations in the gene encoding the α-subunit of the stimulatory G protein ($G_s\alpha$) have reproducibly been found in these neoplasia (2, 3). Although mutations of the menin gene, recently localized in 11q13, probably have little role in pituitary tumorigenesis (4, 5), $G_s\alpha$ is a key element for the activation of the cAMP-dependent pathway that in pituitary cells is a signal for differentiation and proliferation (6). Point mutations that have been first identified in GH-secreting adenomas occur at two specific sites in the $G_s\alpha$ gene, codon 201 or 227, the common effect of both being to constitutively activate adenylyl cyclase by impairing the intrinsic guanosine triphosphatase activity of the subunit (2, 7). The same gain of function mutations have been subsequently identified in a subset of toxic thyroid adenomas and differentiated thyroid adenocarcinomas (8, 9), consistent with the hypothesis that in selected cell types $G_s\alpha$ gene may be converted into an oncogene, designated *gsp* for G_s protein (2, 7, 10–13).

Expression of $G_s\alpha$ can vary over a wide range in human tissues, and several lines of evidence suggest the existence of a direct relationship between cellular $G_s\alpha$ content and activation of the adenylyl cyclase system (14). However, data on $G_s\alpha$ expression in endocrine tumors that depend on cAMP for growth are still limited and controversial. Increased levels of $G_s\alpha$ have been found in tumoral thyroid samples compared with those in normal tissue; the overexpression especially involves the mutant $G_s\alpha$ or is independent of the presence of mutations according to various reports (15–17). Whereas it has been recently suggested that high $G_s\alpha$ levels may be sufficient to stimulate phosphorylation of cAMP response element-binding protein in GH-secreting adenomas (18), the $G_s\alpha$ content in pituitary tumors with and without activating $G_s\alpha$ mutations has been poorly investigated to date. The present study shows extremely low amounts, if any, of $G_s\alpha$ in GH-secreting adenomas carrying $G_s\alpha$ mutations and investigates the molecular mechanisms underlying this phenomenon.

Subjects and Methods

Patients and tumors

The study was carried out on 26 GH-secreting tumors surgically removed by the transsphenoidal route from patients affected with active acromegaly. Acromegaly was diagnosed on the basis of clinical features, elevated insulin-like growth factor I plasma levels and elevated GH levels not suppressible during oral glucose tolerance test. No patient had previously undergone pituitary irradiation. Patients did not receive drugs known to influence G protein expression, in particular ethanol, opiates, and lithium. Small adenoma fragments were fixed for light and

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electron microscopy to check the adenomatous nature of the material, as previously described (19). The remaining tissue was quickly frozen for $G_s\alpha$ gene analysis, adenylyl cyclase activity, and immunoblotting. For cell cultures adenomatous tissues were placed in sterile medium until enzymatic digestion. Local ethical approval was obtained for all studies.

Analysis of mutations in $G_s\alpha$ gene

DNA and ribonucleic acid (RNA) were obtained from tissue homogenates by acid guanidine thiocyanate-phenol-chloroform extraction using commercial kits (Tri-Reagent, Molecular Research Center, Inc., Cincinnati, OH). Analysis of mutations was carried out as previously described (20). Briefly, 100 ng DNA were amplified in a 50-μL reaction mixture containing 2 U *Taq* polymerase (Perkin-Elmer/Cetus, Norwalk, CT), 0.5 μmol/L of each primer, 0.2 mmol/L 2-deoxy-nucleoside-5′-triphosphate, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 0.001% (wt/vol) gelatin [40 cycles of 94 C for 1 min, 56 C (for codon 201 of the $G_s\alpha$ gene) or 54 C (for codon 227 of the $G_s\alpha$ gene) for 1 min, and 72 C for 1 min]. The oligonucleotides used to amplify codon 201 of the $G_s\alpha$ gene were 5′-CCAAACTACTCCAGACCTTT-3′ and 5′-TGGAAGTTGACTTTGTCCAC-3′; the oligonucleotides used to amplify codon 227 of the $G_s\alpha$ gene were 5′-ACAGAGATCATGGTTTCTTG-3′ and 5′-TTAACCAAAGAGAGCAAAGC-3′. Amplified fragments were purified and directly sequenced using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham, Aylesbury, UK).

RT-PCR

Total RNA (500 ng) was reverse transcribed at 42 C for 1 h in the following reaction conditions: 1 mmol/L of each deoxynucleoside triphosphate, 1 U RNAsin, 100 pmol random hexamers, and 200 U reverse transcriptase enzyme. The mixture was then heated at 95 C for 5 min and quick chilled on ice. PCR was performed on the entire complementary DNA (cDNA) product with *Taq* DNA polymerase and specific oligonucleotide primers. Primer sequences used for amplification of human $G_s\alpha$ cDNA were 5′-CATGGGCTGCCTCGGGAA-3′ and 5′-TTAGAGCAGCTCGTACTGAC-3′. PCR conditions were as follow: 94 C for 1 min, 54 C for 1 min, and 72 C for 2 min for 40 cycles. Amplification of the housekeeping gene human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed in all samples studied to verify the integrity of the ribonucleic acid, as previously described (21). An additional control was obtained by omitting the RT step to detect the presence of any contaminating genomic DNA. PCR products were visualized on a 4% agarose gel stained with ethidium bromide. Amplified fragments were purified and directly sequenced using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham).

Semiquantitative RT-PCR

Levels of $G_s\alpha$ RNA transcripts were evaluated by semiquantitative PCR performed using the GAPDH gene as an internal standard. For each cDNA, preliminary experiments were conducted to determine the PCR cycles corresponding to the exponential phase. Therefore, amplifications of GAPDH and $G_s\alpha$ sequences were performed for 23 and 21 cycles, respectively. Oligonucleotide primers were 5′-end labeled with [γ-^{32}P]ATP. PCR products were visualized on a 10% polyacrylamide gel. DNA bands were cut off the gel, and radioactivity was counted in a β-counter. Ratios of radioactivity detected in $G_s\alpha$ DNA fragments and in their correspondent GAPDH DNA fragments were calculated.

Immunoblotting of $G_s\alpha$ protein

Immunoblotting analysis was performed on pituitary tissues as previously described (22). Briefly, tissues were homogenized by a glass-Teflon Potter homogenizer in 10 mmol HEPES (pH 7.3), 150 mmol NaCl, 1 mmol phenylmethylsulfonylfluoride, 4 μg/mL pepstatin, and 4 μg/mL aprotinin. Total homogenates were centrifuged at 3,000 $\times$ *g* for 15 min at 4 C, and supernatants were centrifuged at 40,000 $\times$ *g* for 30 min at 4 C. In some experiments the supernatant fractions were used. Crude membranes were resuspended in solubilization buffer, and protein concentrations were determined using the bicinchoninic acid protein assay. Membrane proteins (20 μg) were separated by 10% SDS-PAGE, electroblotted to nitrocellulose, probed with specific polyclonal antibodies to the G protein α-subunits at 1:250 dilution for 3 h at room temperature, followed by ^{125}I-labeled protein A (4–5 $\times$ 10^5 cpm/mL), and finally subjected to autoradiography with X-Omat x-ray film (Eastman Kodak Co., Rochester, NY). The regions of the blot corresponding to the bands and equivalent sized areas not containing immunoreactive proteins were excised, and radioactivity was quantitated by γ-counter (Packard A5550, Downers Grove, IL). In some experiments detection of $G_s\alpha$ was achieved by using donkey horseradish peroxidase-linked antirabbit IgG followed by densitometer analysis. Two commercial antibodies were used to detect $G_s\alpha$: one directed against a peptide corresponding to residues RMHLRQYELL near the C-terminus of $G_s\alpha$ and the other directed against a peptide corresponding to amino acids 100–119 mapping within the amino-terminal domain. Antibodies directed against the C-termini of $G_{i1/2}\alpha$, $G_{i3}\alpha$, $G_{q/11}\alpha$, and Gβ and the N-terminal sequence of $G_o\alpha$ were used to detect the different subunits. The specificity of the reaction was evaluated as previously described (22).

Cell culture

Tumor fragments were enzymatically dissociated using trypsin and deoxyribonuclease as previously described (23). The cell suspensions obtained largely consisted of single cells with a viability, as assessed by trypan blue exclusion, greater than 90%. The dispersed cells were plated at a density of 2.5 $\times$ 10^5 cell/mL in plastic dishes and cultured in DMEM supplemented with 10% FCS and antibiotics at 37 C in an atmosphere of 95% air-5% CO_2 in a humidified incubator. After 24 h, medium was removed by aspiration, and the cell monolayers were washed twice and incubated with cholera toxin (1 μg/mL for 8 h) or forskolin (10 μmol/L for 1 h) in Hanks' Balanced Salt Solution, 500 μmol/L 3-isobutyl-1-methylxanthine (IBMX) and 0.1% BSA. At the end of incubation, cells were lysed in solubilization buffer and homogenized for immunoblotting as described above.

Adenylyl cyclase assay

The adenylyl cyclase assay was carried out as previously described on crude membrane preparations sedimented from tumor homogenates by centrifugation at 40,000 $\times$ *g* for 30 min. The assay mixture contained 25 mmol/L Tris-HCl (pH 7.4), 500 μmol/L IBMX, 1 μmol/L GTP, 0.2 mmol/L ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 2 mmol/L ATP, 7 mmol/L phosphocreatine, and creatine phosphokinase (20 U/mL). The reaction was initiated by the addition of membranes (0.5 mg protein/mL), and incubation proceeded at 30 C for 8 min. The estimation of the amount of cAMP formed was previously described (24).

Materials

Culture medium, leupeptin, pepstatin, aprotinin, phenylmethylsulfonylfluoride, trypsin, soybean trypsin inhibitor, donkey horseradish peroxidase-linked antirabbit IgG, IBMX, forskolin, and cholera toxin were purchased from Sigma Chemical Co. (St. Louis, MO). The bicinchoninic acid protein assay was purchased from Pierce (Rockford, IL). Nitrocellulose membrane and mol wt standards were obtained from Bio-Rad Laboratories (Hercules, CA). Polyclonal rabbit anti-G protein α-subunit antibodies and [^{125}I]protein A were purchased from New England Nuclear-DuPont (Boston, MA). An additional anti-$G_s\alpha$ serum (amino acids 100–119) and control $G_s\alpha$ were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). X-Omat x-ray film was obtained from Eastman Kodak Co. Antirabbit Ig. biotinylated species-specific whole antibody, and streptavidin Texas Red were obtained from Amersham. All other chemicals were reagent grade.

Results

Analysis of mutations in the $G_s\alpha$ gene

Genomic DNAs from 26 GH-secreting adenomas were analyzed for mutations in codons 201 and 227. Eleven tumors (42%) were found to harbor point mutations of the $G_s\alpha$ gene as follows: nine at codon 201 (CGT>TGT/R201C in eight and CGT>AGT/R201S in one) and two at codon 227

(CAG>CGG/Q227R) and were defined as gsp^+. All mutations reported here are known to constitutively activate adenylyl cyclase. Indeed, adenylyl cyclase activity was 102 ± 19 pmol cAMP/mg protein·min in gsp^+ *vs.* 10.5 ± 2.0 in gsp^- tumors ($P < 0.001$).

Although genomic DNAs from gsp^+ tumors showed both mutant and wild-type alleles, cDNAs obtained by reverse transcribing RNA from the four tumors tested contained very low amounts, if any, of wild-type $G_s\alpha$ sequences, indicating a preponderance of the mutant $G_s\alpha$ transcripts in these tumors (Fig. 1 and data not shown).

Immunoblotting analysis of $G_s\alpha$ in wild-type and mutant GH-secreting adenomas

Figure 2 summarizes the data of immunoblots obtained with antiserum to $G_s\alpha$ in gsp^- and gsp^+ adenomas. In the two groups of tumors the levels of $G_s\alpha$ expression were markedly different. In particular, the two isoforms of $G_s\alpha$ arising from alternative splicing of messenger RNA (mRNA) transcripts were expressed at high levels in all gsp^- adenomas (Fig. 3). In contrast, $G_s\alpha$ was undetectable in blots obtained from eight gsp^+ tumors and barely detectable in the remaining three; the reduction was independent from the location of the mutation (Fig. 3). A similar reduction in $G_s\alpha$ was observed in three gsp^+ tumors using a different antiserum raised against a peptide corresponding to amino acids 100–119 mapping within the amino-terminal domain (data not shown). With the exception of two microadenomas, all tumors were tested at least twice, giving similar results. Data were confirmed by visualizing the reaction with peroxidase-linked antirabbit IgG followed by densitometer analysis (data not shown). Moreover, $G_s\alpha$ was undetectable or detectable at very low levels in the supernatant fractions resulting from the second centrifugation of gsp^+ tumors (data not shown).

gsp^+ tumors showed a selective reduction in $G_s\alpha$. In fact, probing with the antiserum specific for the common carboxyl-terminal sequence of $G_q\alpha$ and $G_{11}\alpha$ detected a single band of approximately 44 kDa that was expressed at high levels in both gsp^- and gsp^+ tumors (Fig. 4). Similarly, using antiserum specific for the β-subunit common to G proteins, all tumors showed high amounts of this protein (Fig. 4). As far as the levels of the G proteins of the G_i family are concerned,

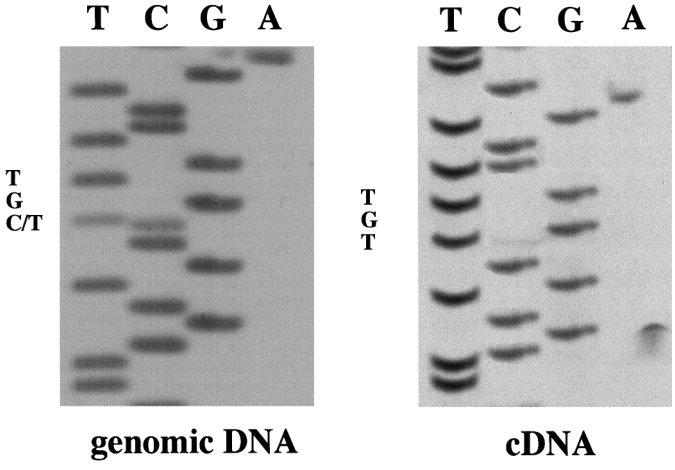

FIG. 1. Direct sequencing of PCR-amplified region surrounding codon 201 in one gsp^+ tumor. Genomic DNA from the tumor showed similar amounts of both wild-type (CGT) and mutant (TGT) sequence, whereas cDNA from the same tumor predominantly showed the mutant nucleotide.

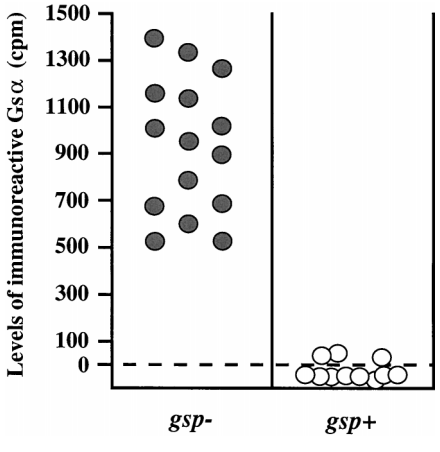

FIG. 2. Levels of $G_s\alpha$ protein in GH-secreting adenomas without (gsp^-; n = 15) and with (gsp^+; n = 11) activating mutations in the $G_s\alpha$ gene. Immunoblotting was performed with antiserum to $G_s\alpha$ (1:250) on 20 μg loaded proteins and was quantitated by measuring the radioactivity of the excised bands corresponding to the blots.

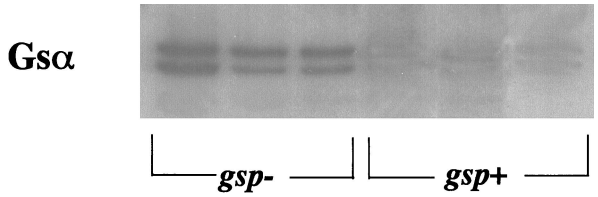

FIG. 3. Representative immunoblotting performed with antiserum to $G_s\alpha$, showing different amounts of the 2 isoforms of $G_s\alpha$. Each line was loaded with 20 μg crude membrane proteins from 3 gsp^- and 3 gsp^+ GH-secreting adenomas. The immunoblots from gsp^- adenomas were representative of the 15 gsp^- tumors. In the remaining 8 gsp^+ adenomas, $G_s\alpha$ isoforms were undetectable (see Fig. 2).

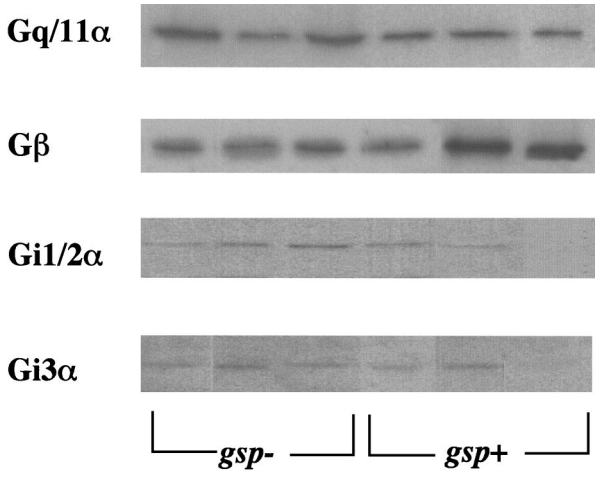

FIG. 4. Representative immunoblotting performed with antisera to $G_{q/11}\alpha$, $G_{i1/2}\alpha$, $G_{i3}\alpha$, and the common β-subunit in membrane preparations from gsp^- and gsp^+ tumors.

$G_{i1/2}\alpha$ and $G_{i3}\alpha$ were detected at very low levels and $G_o\alpha$ was found in significant amounts in both gsp^- and gsp^+ tumors (Fig. 4 and data not shown).

Quantification of $G_s\alpha$ mRNA levels in wild-type and mutant GH-secreting adenomas

As the amounts of tumoral tissue available were too limited to perform Northern blot analysis, we employed semiquantitative RT-PCR to determine levels of $G_s\alpha$ mRNA in GH-secreting adenomas (seven gsp^- and six gsp^+ tumors). Despite the large individual variations, the mean $G_s\alpha$ mRNA levels were similar in wild-type and mutant tissues (target/standard ratio, 0.202 ± 0.150 in gsp^- *vs.* 0.144 ± 0.094 in gsp^+; P = NS), indicating that the low levels of $G_s\alpha$ protein in gsp^+ tumors were not due to a reduction in $G_s\alpha$ transcripts.

Modification of $G_s\alpha$ levels in cultured cells from GH-secreting adenomas

Cells obtained from GH-secreting adenomas were treated with cholera toxin, a toxin known to mimic *gsp* mutations by covalently modifying Arg^{201}. Treatment of cells obtained from three gsp^- tumors with cholera toxin (1 μg/mL) for 8 h caused a marked reduction in$G_s\alpha$ levels, as assessed by immunoblotting analysis (Fig. 5). $G_s\alpha$ levels observed after the treatment were similar to those observed in untreated cells obtained from gsp^+ tumors. In contrast, incubation with forskolin (1 μmol/L) did not reduce $G_s\alpha$ levels, thus ruling out cAMP as a mediator of the toxin-induced decrease in immunoreactive $G_s\alpha$ (data not shown). None of these treatments modified $G_s\alpha$ levels in cells obtained from two gsp^+ tumors (data not shown).

Discussion

The study clearly indicates that GH-secreting adenomas carrying activating mutations of the $G_s\alpha$ gene are characterized by extremely low levels of the mutant protein, thus confirming our previous preliminary data obtained in individual tumors (13). In the present series heterozygous gain of function mutations of the $G_s\alpha$ gene were detected in about a third of GH-secreting adenomas, as reported in previous studies (2, 7, 25, 26); the substitution CGT→TGT at codon 201 was the most frequently observed. By analogy with most dominant oncogenes and in agreement with previous reports (2), the large majority of the $G_s\alpha$ transcript originated from the mutant allele. In fact, although the analysis of tumor genomic DNA revealed equal amounts of mutant and wild-type $G_s\alpha$ sequences, cDNA amplified by PCR from the same tumors almost exclusively showed mutant $G_s\alpha$.

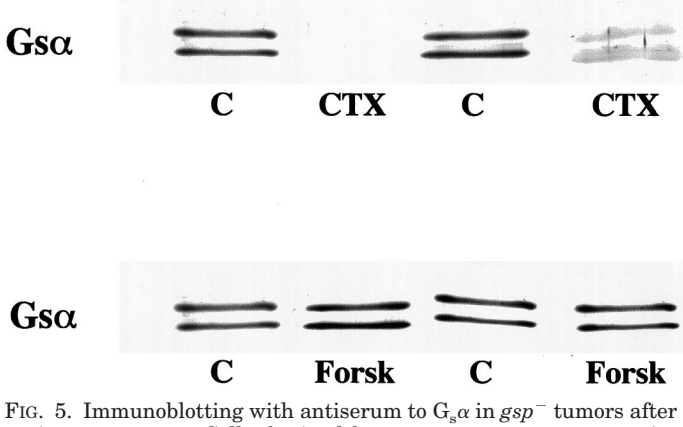

FIG. 5. Immunoblotting with antiserum to $G_s\alpha$ in gsp^- tumors after various treatments. Cells obtained from two gsp^- tumors were maintained without (C) or with cholera toxin (CTX; 1 μg/mL for 8 h) or forskolin (Forsk; 10 μmol/L for 1 h).

Tumors with and without $G_s\alpha$ mutations were markedly different in their content of $G_s\alpha$ protein. All gsp^- tumors showed high levels of the two $G_s\alpha$ isoforms arising from alternative mRNA splicing that, although variable among the different tumors, were similar to those observed in prolactinomas and nonfunctioning adenomas and in the normal pituitary (22) (our unpublished observations). Conversely, in gsp^+ tumors $G_s\alpha$ protein was undetectable or, in a minority of tumors, barely detectable; this pattern was in contrast with the overexpression that characterizes the common oncogenes. The reduction in $G_s\alpha$ content was a general phenomenon, being present in all gsp^+ tumors independently from the location of the mutation, and a selective event, as the other G proteins were present in similar amounts in gsp^+ and gsp^- tumors. The reduction in $G_s\alpha$ levels obtained with an antiserum directed against a sequence in the C-terminus of $G_s\alpha$ was confirmed using a different antiserum recognizing amino acids within the amino-terminal domain. The parallel results with two antibodies directed against spatially separated portions of $G_s\alpha$ polypeptide make it unlikely that mutations at codons 201 and 227 somehow prevented the antibodies from detecting the mutant protein. Although these antibodies did not discriminate between wild-type and mutant proteins, the reduction of $G_s\alpha$ in gsp^+ tumors probably affected the mutant protein because these tumors mainly contained mutant $G_s\alpha$ transcripts.

The biological mechanisms responsible for the low amount of mutant $G_s\alpha$ protein in gsp^+ tumors were further investigated. Using RT-PCR analysis to monitor endogenous $G_s\alpha$ steady state mRNA levels, no significant difference in $G_s\alpha$ mRNA levels between gsp^+ and gsp^- tumors was detected, thus ruling out that reduced mRNA synthesis or stability might be responsible for the low expression of $G_s\alpha$ in gsp^+ tumors.

Experiments were carried out by treating gsp^- tumors with cholera toxin, a toxin known to mimic *gsp* mutations by covalently modifying Arg^{201} and to constitutively activate adenylyl cyclase. The toxin was able to dramatically reduce $G_s\alpha$ protein levels; the resulting phenotype was superimposable to that characterizing gsp^+ tumors. These data are in agreement with previous studies showing that several hours of exposure to cholera toxin leads to a progressive $G_s\alpha$ disappearance from GH_3 pituitary cells and S49 lymphoma cells (27, 28). Similarly, low levels of $G_s\alpha$ were observed in S49 cyc^- cells expressing mutant $G_s\alpha$ (R201C) (28). In these experimental models a conformational change that loosens $G_s\alpha$ attachment to membranes and increases its degradation rate has been proposed to occur. Accelerated degradation of mutant $G_s\alpha$ in pituitary adenomas is consistent with the lack of difference in $G_s\alpha$ mRNA levels between gsp^- and gsp^+ tumors and with the absence of detectable $G_s\alpha$ in the cytosolic fractions from gsp^+ tumors. Taking into account that both cholera toxin and *gsp* mutations activate adenylyl cyclase by

inhibiting $G_s\alpha$ guanosine triphosphatase and thereby preventing the formation of the inactive $\alpha\beta\gamma$ complex, it is conceivable that in the activated free state, α-subunit is highly susceptible to degradation (27, 28).

The low amounts of $G_s\alpha$ found in pituitary tumors is in contrast with previous reports showing that thyroid toxic adenomas and papillary and follicular carcinomas bearing $G_s\alpha$ mutation at codon 201 contain high amounts of the corresponding protein (15, 16). As it seems unlikely that the rates of degradation of mutant $G_s\alpha$ protein may be different in thyroid and pituitary tissues, it is possible to hypothesize that thyroid tumors contain both wild-type and mutant transcripts, and that the immunoreactive protein found in these tumors is the stable wild-type $G_s\alpha$. However, this hypothesis needs to be supported by experimental evidence.

As observed in cells treated with cholera toxin, gsp^+ tumors showed high adenylyl cyclase activity, suggesting that the concentrations of $G_s\alpha$ are substantially higher than that required for activation of adenylyl cyclase. However, several lines of evidence support the idea that the amount of available G_s is important for stimulation of cAMP synthesis. In fact, in cells transfected with cDNA for $G_s\alpha$ there is a close relationship between $G_s\alpha$ levels and adenylyl cyclase activation (14). Moreover, it is well known that heterozygous loss of function mutations of $G_s\alpha$ causes the resistance to PTH and other hormones that characterizes pseudohypoparathyroidism type I, indicating that G_s limits the maximal activity of adenylyl cyclase (29).

Several mechanisms able to contrast the consequence of the constitutive activation of the cAMP-dependent pathway have been recently identified in gsp^+ GH-secreting adenomas. In particular, it has been proposed that the high sensitivity to the inhibitory action of somatostatin and the increased degradation of cAMP caused by phosphodiesterase overactivity may be involved in determining the *in vivo* phenotype of *gsp* mutations (12, 20, 26). The low content of mutant $G_s\alpha$, probably due to its accelerated removal, may represent an additional mechanism of feedback response to the constitutive activation of cAMP signaling in pituitary tumors carrying gain of function mutations of $G_s\alpha$ gene.

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